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‘Disease Management and Growth Promotion in Tomato (*Solanum lycopersicum* L.) through Application of Fungal Bio-Agents’

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Introduction

Tomato (*Solanum lycopersicum*) is one of the most widely cultivated vegetables worldwide, valued both for its nutritional benefits and its economic importance (Gerszberg *et al.,* 2015; Kumar *et al.,* 2021). Tomato is nutritionally very important and has several medicinal uses. Tomato is low in fat and hence used for all diabetic preparations and incorporated in weight reduction plans. It is also rich in amino acids and it acts as a source of many minerals. It is unique in having lycopene pigment along with vitamin A, C and E (Deshpande *et al.,* 2017). Tomatoes are grown worldwide in countries such as China, India, Turkey, United States, Egypt, Italy, and Mexico. India ranks second in the production of tomatoes, producing over 20 megatonnes of tomatoes in the year 2022-23. According to the Department of Agriculture and Farmers' Welfare, latest estimates, India's tomato production has grown by 4% in 2023 – 2024, reaching 213.20 lakh tonnes. Maharashtra is in seventh position in Tomato production in India. Maharashtra produces 1332.09 Kilotonnes of tomatoes in the year 2023-24 in an area of 552.3 km². Maharashtra accounts for 6.2% of total tomato production in India. (https://knowledge.desikheti.com/top-10-tomato-producing-states-in-india-a-detailed-statewise-overview/). However, its cultivation is often severely affected by various biotic stresses, primarily diseases caused by fungi, bacteria, and viruses. Among fungal diseases, seedling Damping off, septoria leaf spot, Eary blight, Late blight, Anthracnose, Powdery mildew, southern leaf blight, Fusarium wilt, Verticillium wilt, Buckeye rot are particularly notorious for causing significant yield losses (Kumar *et al.,* 2018; Ewekeye *et al.,* 2021; Panno *et al.,* 2021; Pandey *et al.,* 2022; Shahid *et al.,* 2022).

In recent years, the overuse of chemical pesticides has led to concerns about environmental sustainability, food safety, and the development of pathogen resistance (Zhou *et al.,* 2024). In this context, the exploration of alternative, eco-friendly disease management strategies have become increasingly important. Fungal bioagents, particularly *Trichoderma*, are identified as the genus with greatest potential comprising 25 biocontrol agents that have been used against a number of plant fungal diseases. In addition to *Trichoderma*, nine genera are recognized as significant comprising five or more known antagonistic species, namely, *Alternaria, Aspergillus, Candida, Fusarium, Penicillium, Pichia, Pythium, Talaromyces, and Verticillium*, have shown great promise in managing plant diseases and promoting growth (Thambugala *et al.,* 2020). These bioagents not only act as biocontrol agents against soil-borne pathogens but also enhance plant growth through mechanisms such as improved nutrient uptake, growth hormone production, and stress tolerance. This research aims to investigate the potential of fungal bioagents in managing tomato diseases while promoting growth, ultimately leading to a more sustainable approach to tomato farming.

Review of Literature:

The tomato crop is highly vulnerable to both abiotic and biotic stresses throughout its growth cycle, from seedling to harvest. These stresses include extreme temperatures, excessive moisture, environmental pollution, insect pests, and a wide range of diseases. Tomato plants are significantly impacted by several major diseases caused by fungi, bacteria, viruses, and nematodes (Sharma *et al.,* 2021). Globally, tomatoes are affected by over 200 known diseases (Atherton & Rudich, 1986).

Fungal diseases, including Alternaria blight (*Alternaria alternata*), early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), collar rot (*Sclerotium rolfsii*), and damping-off (*Pythium* sp.), cause significant yield losses in tomato crops (Sharma *et al.*, 2021).

Among these, Alternaria blight caused by *A. alternata* is one of the most destructive and frequently occurring diseases, leading to substantial crop damage worldwide (Sharma *et al.,* 2021). This disease has become a critical limiting factor in tomato cultivation, with increasing severity reported at 49.5% (Abhinandan *et al.,* 2004) and fruit yield losses ranging from 47.60% to 79.00% (Saha & Das, 2012; Adhikari *et al.,* 2017).

Another disease known as Fusarium vascular wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici*, is also highly destructive disease of tomatoes, affecting crops in both field and greenhouse conditions. It results in yield losses of 45–55%, which can increase to 70% (Panno *et al.,* 2021)under favourable conditions with temperatures of 27–30°C. Srinivas et al., (2019) reported that this disease can severely impact tomato yields, potentially causing maximum losses. This disease blocks the xylem, disrupting water transport in the plant (Ponsankar *et al.,* 2023). Affected plants show symptoms such as wilting, yellowing of leaves, and necrotic roots, along with discoloration of vascular tissues (Pandey *et al.,* 2023). *Fusarium oxysporum* f. sp. *lycopersici* spreads through irrigation water and infected planting materials and can persist in contaminated soil for decades (Ma *et al.,* 2023).

Preventing crop losses of tomato caused by pathogenic fungi, bacteria, viruses, viroids, and nematodes is a critical challenge for achieving economically viable plant production. Currently, the rapid and effective control of fungal and other crop diseases primarily relies on synthetic pesticides. These chemical fungicides, both systemic and non-systemic, play a major role in disease management. However, their use has several drawbacks, including environmental harm, toxic residues in crops that pose risks to human health, and high costs. Additionally, microorganisms may develop resistance to these pesticides over time, further complicating their effectiveness (Sharma *et al.,* 2021).

This study focuses to manage Alternaria blight and Fusarium wilt diseases in tomato using various eco-friendly fungi. Extensive literature is available on the mycological approaches to Alternaria blight and Fusarium wilt management, including the following:

The use of fungal bioagents in agriculture has gained considerable attention due to their multifaceted role in disease management and growth promotion. According to recent studies, *Trichoderma spp.* is among the most widely used bioagents for biocontrol, due to their ability to suppress soil-borne pathogens through mechanisms like mycoparasitism, production of antibiotics, and competition for nutrients (Harman *et al.,* 2004). *Trichoderma* is a hemibiotrophic fungus known for its ability to reduce the severity of plant diseases through various mechanisms. It employs antagonism and mycoparasitism to directly attack or inhibit the growth of plant pathogens. Additionally, it enhances plant defense by inducing both systemic and localized resistance (Fontenelle *et al.,* 2011).

Numerous studies have documented the beneficial effects of *Trichoderma* species e.g., *Trichoderma harzianum* isolate 2413, as cited by Benítez *et al.,* (2004), has been shown to promote the growth of tomato, tobacco, and cotton plants while providing protection against various pathogens. Ramírez-Cariño *et al.* (2020) investigated the effectiveness of potential biocontrol agents (PBAs), *Bacillus paralicheniformis* and *Trichoderma*

asperelloides, both in vitro and in vivo, for managing two major tomato diseases: vascular wilt caused by *Fusarium oxysporum* and early blight caused by *Alternaria alternata*. The effectiveness of individual and combined PBA treatments against *Fusarium oxysporum* and *Alternaria alternata* infections was assessed in tomato plants. In vitro tests revealed that *Trichoderma asperelloides* demonstrated a strong mycoparasitic effect on both pathogens, employing mechanisms such as hyphal strangulation and penetration. Additionally, individual PBA treatments effectively controlled *Alternaria alternata* and *Fusarium oxysporum* in tomato plants, reducing disease severity by over 53.8% and 66.7%, respectively.

In another study conducted by Philip *et al.,* (2024) explored the efficacy of *Trichoderma* isolates in combating tomato leaf spot disease, primarily caused by *Alternaria alternata*. Six *Trichoderma* isolates were assessed for their ability to inhibit Alternaria hyphal growth using dual culture, ethyl acetate extract, and volatile organic compounds (VOCs) techniques. Out of six isolates, the most promising biocontrol isolate was identified as *T. afroharzianum* isolate TRI07 which produced 34 compounds were identified, with major components including 1-octen-3-one, ethanol, and hexanedioic acid, bis(2-ethylhexyl) ester to tackle the *Alternaria alternata*.

Objectives of research:

Given the significance of tomatoes and the impact of vascular wilt caused by *Fusarium oxysporum* and blight caused by *Alternaria alternata* on productivity, the following objectives have been set to investigate the problem in detail and identify management strategies for controlling *Alternaria alternata*, *Fusarium oxysporum* f. sp. *lycopersici*, with the aim of improving tomato yield in a cost-effective and eco-friendly manner:

1. Isolation of phytopathogen of Tomato (*Solanum lycopersicum*) most common in Marathwada region of Maharashtra and its pathogenicity.
2. To isolate and evaluate the effectiveness of different fungal bioagents in controlling major tomato diseases caused by fungal pathogens like *Fusarium oxysporum* f. sp. *lycopersici* and *Alternaria alternata*.
3. Identification of Efficient fungal bioagents.
4. To find out the mechanism of Bio-control agents for management of tomato phytopathogen.
5. To formulate the fungal bioagents and to evaluate it for maximum effectiveness in disease management in vitro conditions.
6. To assess the growth-promoting effects of fungal bioagents on tomato plants.

Hypothesis:

Traditionally, farmers use chemicals like fungicides and bactericides to protect crops from diseases. While chemical methods are effective for managing crop diseases, they can also harm the environment. The proposed idea focuses on controlling plant diseases with naturally occurring beneficial microorganisms, such as *Trichoderma* species. This method is commonly known as biocontrol of plant pathogens.

Biocontrol using fungal agents is an eco-friendly and cost-effective method for managing plant pathogens, providing a sustainable alternative to chemical control. Given the

importance of environmentally safe and targeted solutions, there is a growing need to explore such methods for managing tomato diseases effectively.

Research Methodology:

To resolve this problem, the following methodology will be adopted:

1. **Isolation and Identification of Phytopathogens and its pathogenicity**:
   For isolation of Phytopathogen, infected parts of tomato will be collected from different fields and brought to laboratory in polythene bags. Isolation of Phytopathogens will be carried out by method used by (Rakh, 2010) from infected stem and infected leaves. Pathogenicity test will be carried out to prove the Koch's postulates. Fungal phytopathogens will be identified by using the book Subramanian (1971) and molecular techniques.
2. **Isolation of** Fungal bioagents **from rhizospheric soil and its screening for inhibition of** *Fusarium oxysporum f. sp. lycopersici and Alternaria alternata* **by In Vitro method**:
   For isolation of rhizospheric fungal bioagents, rhizospheric soils from different fields will be collected in polythene bags and brought to laboratory. Isolation of Fungal spp. will be carried out by using methods followed by Nor, and Norazwina, (2018) and its screening for efficient biocontrol will be done by method of Rakh *et al.,* (2010).
3. **Identification of Efficient fungal bioagents:**
   Identification of efficient fungal bioagents will be carried out by morphological, cultural biochemical characteristics and molecular techniques.
4. **Mechanism of fungal bioagents for management of tomato phytopathogen:**
   To illustrate the biocontrol mechanism, the efficient fungal bioagents will be tested to produce antibiotics, mycoparasitism, and cell wall degrading enzymes (Naher, et al., 2014).
5. **Formulation of the fungal bioagents and evaluation it for maximum effectiveness in disease management in vitro conditions:**
   Carrier based powder formulation (CBPF) of Fungal bioagents will be prepared and used for the Pot Assay, to test the biocontrol ability of selected fungal bioagents (Rakh *et al.,* 2022).
6. **Assessment of the growth-promoting effects of fungal bioagents on tomato plants:**
   Growth promoting ability of Fungal bioagent will be analysed by direct inoculation in Pot and studying different parameters (Rakh *et al.,* 2022).

Importance of Study:

Importance of this research lies in the fact that tomato growing farmers will get alternative to the chemical fungicides that being used to manage the phytopathogens of tomato. Still lot of study need to be done in relation to commercial production of biocontrol agents.

Internation Status:

Tomatoes are a globally significant crop, grown extensively for fresh consumption and processing. However, tomato plants are vulnerable to numerous pathogens, including fungal, bacterial, and viral diseases, such as *Alternaria solani*, *Fusarium oxysporum*, and *Ralstonia solanacearum*. Biocontrol methods, involving the use of beneficial microorganisms, are emerging as sustainable alternatives to chemical pesticides. Here is some research conducted by several researchers in relation to biocontrol and growth promotion of Tomato:

Attia *et al.,* (2022) evaluated Four Plant growth-promoting fungi (PGPF) identified genetically as *Aspergillus flavus, Aspergillus niger*, *Mucor circinelloides and Penicillium oxalicum*, for their antifungal activity against *Fusarium oxysporum* in vitro, showing promising results with a minimum inhibitory concentration (MIC) ranging from 0.25 to 0.5 mg/mL. The study also examined physiological markers in plants related to induced systemic resistance (ISR). The findings revealed that *A. niger*, *M. circinelloides*, *A. flavus*, and *P. oxalicum* significantly reduced disease severity by 16.60%, 20.83%, 37.50%, and 45.83%, respectively. Additionally, they provided high protection percentages of 86.35%, 76.87%, 56.87%, and 59.06%, respectively.

With another study conducted by Abdelaziz *et al.,* (2022) revealed that Ethyl acetate extracts of *A. flavus* MZ045563, *A. fumigatus* MZ045562 and *A. nidulans* MZ045561 exhibited antifungal activity toward *F. oxysporum* where inhibition zone diameters were 15, 12 and 20 mm, respectively. Moreover, extracts of all fungal isolates at concentration 7.5 mg/mL reduced conidia germination from 94.4 to 100%. *Fusarium* infection caused a destructive effect on tomato plant, high severity disease index 84.37%, reduction in growth parameters, photosynthetic pigments, and soluble protein.

Though the work on biocontrol of tomato phytopathogens and growth promotion of tomato plant is in progress internationally, but it must intensified further at level of commercialization.

National Status:

India is a significant producer of tomatoes, contributing to global supply both for domestic consumption and export. However, tomato cultivation in the country faces challenges from various phytopathogens, including fungi (*Alternaria solani*, *Fusarium oxysporum*), bacteria (*Ralstonia solanacearum*, *Xanthomonas campestris*), and viruses (Tomato Leaf Curl Virus - ToLCV). To manage these diseases various fungicides being utilized which also causes environmental and human health problems, so to find an alternative work on biocontrol of phytopathogens of tomato must be intensified. Some of the work carried out on this issue have been represented here:

Jamil et al., (2021) assessed the efficacy of biocontrol agents *Trichoderma harzianum, T. viride* and *Aspergillus niger* against wilt fungus. All biocontrol agents produced significant inhibitory effect on pathogen development. *T. viride* caused maximum reduction (83.56%) in

growth and fresh (332.49 mg) and dry weight (52.19 mg) of the pathogen. In pot trial seed treatment with *T. viride* caused minimum disease severity (1.2) and maximum significant increase in plant fresh (60.23 g) and dry weight (23.82 g), plant length (63.91 cm) and yield/plant (258.05 g). *T. harzianum* seed treatment showed highest increase in chlorophyll (3.04 mg/g fresh weight) and lycopene content (75.43 mg/kg fresh weight) whereas *T. viride* seed treatment showed maximum total phenols (93.58 µg catechol/g fresh weight).

In another significant study conducted by Pothiraj et al. (2021) conducted pot experiments under polyhouse conditions to evaluate the efficacy of a talc-based biocontrol formulation containing *Pseudomonas putida* (TEPF-Sungal-1), *Trichoderma harzianum* (C S17TH), and *Chaetomium globosum* (C CG-A) against *Fusarium oxysporum* f. sp. *lycopersici* (Fol). The biocontrol treatment, applied as a seed dressing and soil application, was as effective as cell suspensions and performed better than the chemical fungicide mixture (copper oxychloride at 0.25% and carbendazim at 0.1%). It significantly promoted crop growth by 52.3% and reduced vascular wilt incidence by 75%, even under the challenge of a highly pathogenic TOFU-IHBT strain.

All the research work is related to Fusarium wilt of tomato but not much work has been reported in the literature on biocontrol of Alternaria blight of tomato.

Significance of the study:

To enhance productivity in terms of both the quality and quantity of tomato and meet the growing demands of the increasing population, farmers often rely on synthetic agrochemicals to control phytopathogens affecting the crop. However, this practice has resulted in several environmental issues, including: i) Disruption of the soil's ecological balance, ii) Groundwater contamination, iii) Development of pathogen resistance to synthetic chemicals, and iv) Significant health risks to non-target species, including humans.

Therefore, there is a need for sustainable and reliable solutions to address the problem of synthetic agrochemical use in agriculture for controlling phytopathogens. This research aims to explore the use of target-specific rhizospheric microorganisms in particular fungal biocontrol agents for the effective and eco-friendly management of the phytopathogen causing Alternaria blight and Fusarium wilt in tomato, providing a cost-effective alternative.

This research is significant because it offers tomato growers an alternative method for managing Alternaria blight and Fusarium wilt disease. In addition to controlling diseases, microbial control agents promote plant growth without additional fertilizers, thus reducing fertilizer inputs.

Plan of work (Time schedule of activity):

Sr. No.	**Duration** (Month)	**Work to done**
1.	6 months	Isolation and Identification of Phytopathogens and its pathogenicity
2.	12 months	Isolation of Fungal bioagents from rhizospheric soil and its screening for inhibition of *Fusarium oxysporum f. sp. lycopersici and Alternaria alternata* by In Vitro method
3.	18 months	Isolation of Fungal bioagents from rhizospheric soil and its screening for inhibition of *Fusarium oxysporum f. sp. lycopersici and Alternaria alternata* by In Vitro method
4.	24 months	Identification of Efficient fungal bioagents; Mechanism of fungal bioagents for management of tomato phytopathogen
5.	30 months	Formulation of the fungal bioagents and evaluation it for maximum effectiveness in disease management in vitro conditions
6.	36 months	Assessment of the growth-promoting effects of fungal bioagents on tomato plants

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